PERGAMON

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Soil Biology & Biochemistry 35 (2003) 1027–1037

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Soil microbial community characteristics along an elevation gradient in the Laguna Mountains of Southern California

Harold P. Collins[a,*], Michel A. Cavigelli[b]

[a]*Vegetable and Forage Research Unit, USDA-ARS, 24106 North Bunn Road, Prosser, WA 99350, USA*
[b]*Sustainable Agricultural Systems Laboratory, USDA-ARS, Beltsville, MD 20705, USA*

Received 8 March 2002; received in revised form 6 November 2002; accepted 20 January 2003

Abstract

We sampled soil at four sites in the Laguna Mountains in the western Sonoran Desert to test the effects of site and sample location (between or beneath plants) on fatty acid methyl ester (FAME) and carbon substrate ulilization (Biolog) profiles. The four sites differed in elevation, soil type, plant community composition, and plant percent cover. Soil pH decreased and plant density increased with elevation. Fertile islands, defined as areas beneath plants with greater soil resources than bare areas, are present at all sites, but are most pronounced at lower elevations. Consistent with this pattern, fertile islands had the greatest influence on FAME and Biolog profiles at lower elevations. Based on the use of FAME biomarker and principal components analyses, we found that soil microbial communities between plants at the lowest elevation had proportionally more Gram-negative bacteria than all other soils. At the higher elevation sites there were few differences in FAME profiles of soils sampled between vs. beneath plants. Differences in FAME profiles under plants among the four sites were small, suggesting that the plant influence per se is more important than plant type in controlling FAME profiles. Since microbial biomass carbon was correlated with FAME number ($r = 0.85$, $P < 0.0001$) and with FAME named ($r = 0.88$, $P < 0.0001$) and total areas ($r = 0.84$, $P < 0.0001$), we standardized the FAME data to ensure that differences in FAME profiles among samples were not the result of differences in microbial biomass. Differences in microbial substrate utilization profiles among sampling locations were greatest between samples taken under vs. between plants at the two lower elevation sites. Microbial substrate utilization profiles, therefore, also seem to be influenced more by the presence of plants than by specific plant type.
Published by Elsevier Science Ltd.

Keywords: Biolog; C-, N-mineralization; Desert soils; Fatty acid methyl ester; Fertile islands; Microbial biomass

1. Introduction

Spatial variability of soil resources in desert ecosystems is largely controlled by the spatial organization of perennial plants, which create relatively stable 'fertile islands' (Charley and West, 1975; Crawford and Gosz, 1982; Parker et al., 1984; Noy-Meir, 1985), areas under plant canopies that have greater soil organic C and N, C- and N-mineralization potential, soil moisture, and reduced daytime temperatures due to litter accumulation and root influences (Garner and Steinberger, 1989; Kieft et al., 1998; Schlesinger and Pilmanis, 1998). These patterns of nutrient heterogeneity in deserts are controlled primarily by the presence of plant cover rather than by the species forming that cover (Xie and Steinberger, 2001). As rainfall in deserts increases, plant community composition changes, plant density increases, and differences in soil resources between vs. under plants are likely to decrease.

Because of these obvious patterns of long-term direct and indirect plant effects in desert soils, deserts provide an ideal laboratory for studying plant effects on soil microbial community structure and function in situ. The large differences in soil resource levels and environmental conditions between vs. under plants likely select for different soil microbial communities between vs. under plants, but there is currently no evidence of such patterns. Since ecosystem process rates are much higher in islands of fertility compared to adjacent bare areas, differences in microbial community structure between these two areas might also be associated with differences in microbial community activity. In addition, deserts may be ideal locations for studying the effects of plants on soil microbial

* Corresponding author. Tel.: +1-509-786-9250; fax: +1-509-786-9277.
E-mail address: hcollins@pars.ars.usda.gov (H.P. Collins).

0038-0717/03/$ - see front matter Published by Elsevier Science Ltd.
doi:10.1016/S0038-0717(03)00145-7

communities because desert soils tend to have low clay and organic matter contents. Clay and organic matter can interfere with the extraction steps inherent to most molecular methods of determining microbial community structure and with the determination of carbon substrate utilization profiles.

Two relatively simple methods, fatty acid methyl ester (FAME) analysis and Biolog substrate analysis (Biolog Inc.; Garland and Mills, 1991), can be used to characterize soil microbial communities. For example, Cavigelli et al. (1995) showed that spatial variability of some FAMEs exists at the same scale as would be expected for rhizosphere influences in a corn field. Zak et al. (1994) used Biolog to show that functional diversity of bacterial communities varied in conjunction with plant communities along an elevational gradient in the Chihuahuan Desert. Buyer and Drinkwater (1997) showed that soil microbial community structure using FAME profiles and soil microbial community function, using Biolog plate, were both affected by management history in agricultural fields but that changes in community structure were not always accompanied by changes in community function. Siciliano et al. (1998), Ibekwe and Kennedy (1999), Fang et al. (2001) showed, using FAME and Biolog, respectively, that different plant species and/or varieties select for different microbial community structure and function. Buyer et al. (2002), however, used FAME and Biolog to show that soil type, more than plant type, influences microbial community structure and function.

Our purpose in this study was to compare microbial communities at four sites along an elevation gradient in the western Sonoran Desert in Southern California. We compared FAME profiles and Biolog patterns to characterize soil microbial communities. We tested two hypotheses: in desert soils, (1) the extent of differences in FAME and Biolog profiles between vs. under plants will decrease with elevation as the fertile island effect decreases, and (2) differences in FAME and Biolog patterns between vs. under plants will be greater than differences under plants among sites (i.e. presence/absence of plants influences microbial community FAME and Biolog profiles more than plant species).

2. Materials and methods

2.1. Study area

The study area was located in the western extent of the Lower Colorado subdivision of the Sonoran Desert in Southern California (MacMahon and Wagner, 1985). We sampled four sites in May 1994 along a transect running from Ocotillo (152 m elevation) to Mt. Spring (608 m) to Jacumba (1216 m) to Laguna Summit (1950 m) on the eastern slope of the Laguna Mts. Dominant vegetation at Ocotillo was creosote bush (*Larrea tridentata*, (DC) Cov.) and saltbush (*Atriplex spp.*, L.) with approximately 20% of the area covered by vegetation; at Mt. Spring, cheesebush (*Hymenoclea salsola*, A. Nels.), ocotillo (*Fouquieria splendens*, Engelm.) and a variety of *Euphorbia* and *Cactaceae spp.* covered approximately 40% of the area; at Jacumba, chamise (*Adenostoma fasciculatum*, Eastw.) was dominant and vegetation covered about 60% of the area; and at Laguna Summit, red shank (*Adenostoma sparsifolium*, Torr.), and manzanita (*Arctostaphylos spp.*, Parry) formed a closed canopy.

At each site, we established a transect in a random direction and took a pair of soil samples at three points along the transect where the transect intersected a dominant plant species. We took three soil cores from the area directly beneath the plant and three cores in an adjacent open area 90° from and within 2 m of the transect. Each set of three cores was composited to form a single sample. Thus, we collected a total of 24 soil samples, three samples collected under plant canopies (V) and three samples collected from areas between plants, not under plant canopies (B) at each site.

2.2. Soil pH and resources

We determined total soil C and N of each sample in duplicate by dry combustion on a Carlo Erba CHN analyzer Model 1104 (Carlo Erba Instruments, Milano, Italy). Each bulk soil sample was tested for the presence of inorganic C by adding 4 M HCl dropwise to a finely ground sample and observing the degree of effervescence. None of the samples effervesced. We determined soil texture using the hydrometer method (Gee and Bauder, 1986) and measured soil pH using a 1:2 soil/water ratio on duplicate 10 g subsamples. Field capacity for incubations (below) was estimated for each soil type by a volumetric soil water method described by Elliott et al. (1994). Briefly, dried sieved soils were packed into 50 cm^3 graduated cylinders and enough water was added to wet approximately half of the soil in the cylinder. After allowing 18 h for water diffusion we removed the wet soil and determined its moisture content gravimetrically.

Soil microbial biomass C and N (MBC and MBN, respectively) were estimated using the chloroform fumigation–incubation method (Jenkinson and Powlson, 1976). Prior to fumigation, each 25 g sample was pre-incubated at field capacity for 7 d at 25 °C to normalize the vagaries of sample handling, sieving and wetting. We fumigated half the samples and incubated both fumigated soils and non-fumigated control soils in sealed containers at 25 °C for an additional 10 d. We then measured headspace CO_2 in each vial to use in MBC calculations. Carbon dioxide was determined using a Beckman Model 865 infrared gas analyzer (Beckman Instruments, Fullerton, CA). We calculated MBC from the expression, $MBC = (CO_{2(F)} - CO_{2(NF)})/k_C$, where $CO_{2(F)}$ is the quantity of CO_2 evolved from the fumigated sample in the 10 d following incubation,

$CO_{2(NF)}$ is the amount of CO_2 produced from the non-fumigated sample for the same period, and k_C is the proportion of microbial-C mineralized to CO_2, taken as 0.45 (D. Harris, Pers. comm.). We measured soil NH_4 in subsamples before incubations and in samples after incubation to measure MBN. We extracted soil inorganic N using 1 M KCl and measured NH_4 in the extracts using automated colorimetric analysis (Alpkem, 1992) on an Alpkem 500 Series auto-analyzer (Perstorp Analytical Instruments, Williamsburg, OR). We calculated MBN using a similar equation used to that of MBC, substituting NH_4–N release in the place of CO_2 produced and a coefficient, $k_N = 0.4$.

We measured mineralizable soil C by incubating duplicate 25 g soil samples in 160 ml gas-tight bottles at 25 °C for 100 d. We measured CO_2 at 3–5 d intervals during the first 35 d of incubation and then at approximately 7 d intervals. Following each analysis, samples were returned to ambient CO_2 by degassing with compressed air. We report CO_2 evolved at 35 and 100 d of incubation (C_{min35} and C_{min100}, respectively). Soil N-mineralization (N_{min}) was determined by incubating duplicate 10 g soil samples in 60 ml bottles for 35 d at 25 °C. Initial and final NO_3–N and NH_4–N were measured following extraction as for MBN described above.

2.3. Microbial community analyses

We extracted FAMEs from soil subsamples (1 g) according to the standard protocol of the Microbial Identification System (MIS, Microbial ID Inc., Newark, DE, USA) adapted for soil samples (Cavigelli et al., 1995; Sinsabaugh et al., 1999). Briefly, lipids in soil samples were saponified in 5 ml of 3.25 M NaOH in methanol and heated in a 100 °C water bath for 30 min. The FAMEs were extracted by adding a 1.5 ml mixture of 1:1 methyl-*t*-butyl ether (MTBE) and hexane (v:v) and mixed on a rotary shaker for 10 min. The organic phase was then transferred to another ashed test tube and washed with 3 ml of dilute NaOH. The washed organic phase was dried under a flow of nitrogen, resuspended in 0.5 ml of MTBE, then transferred to a gas chromatography vial. Samples were analyzed on a HP 5890 GC equipped with an HP Ultra 2 capillary column (cross-linked 5% Ph Me silicone, 25 m × 0.2 mm × 0.33 mm film thickness) and a flame ionization detector.

The MIS system uses an external calibration standard developed and manufactured by Microbial ID, Inc. (Sasser, 2001). The standard is a mixture of the straight-chained saturated fatty acids from 9 to 20 carbons in length (9:0 to 20:0) and five hydroxy acids. All compounds are added quantitatively so that the gas chromatographic performance can be evaluated by the software each time the calibration mixture is analyzed. We ran the calibration standard every 10–20 samples. Retention time data obtained from injecting the calibration mixture are converted to Equivalent Chain Length (ECL) data for bacterial fatty acid naming. The ECL value for each fatty acid is derived as a function of its elution time in relation to the elution times of the known series of straight chain fatty acids in the calibration mix. Results are reported using standard FAME nomenclature. Interpretation of the FAME profiles was aided by the use of FA markers, those fatty acids which tend to be found in greater quantities in particular groups of organisms (White, 1983; Harwood and Russell, 1984; Vestal and White, 1989; Cavigelli et al., 1995; Zelles, 1999). We also grouped FAME data into fractions as suggested by Zelles (1999) for phospholipid fatty acids (PLFAs).

Whole soil microbial communities were tested for aerobic utilization of 95 different carbon substrates using a commercially available microtiter plate test system designed for use with Gram-negative bacteria (Biolog Inc., Hayward, CA). The system consists of a 96-well microtiter plate. Each well contains a different carbon substrate (plus one negative control with no carbon substrate) and a redox dye (tetrozolium violet) that is reduced to formazan during respiratory activity. Insoluble formazan, a purple-colored compound, accumulates in the cells and its presence is detected with spetrophotometry. We added soil (1 g) to 99 ml of 10 mM phosphate buffer in a sterile bottle and shook the bottle for 20 min. We then chemically flocculated soil particles using $CaCO_3$ and $MgCl_2$ and we transferred aliqouts of supernatant into microplate, which were incubated at 31 °C for 72 h. For statistical analyses we grouped carbon substrates into nine groups.

2.4. Statistical analyses

All analyses were conducted on 23 samples since one sample taken between plants at Laguna Summit was lost. We used separate two-way analyses of variance to determine the effect of site and plant presence on each of the physical, chemical, and biogeochemical parameters measured. These analyses were conducted using the GLM procedure of SAS version 8.0 (SAS Institute, 2001). We used principal components analysis (PCA) to describe multivariate Biolog, FAME, and biogeochemical data sets in two dimensions. Reducing the number of variables by grouping FAMEs or Biolog substrates is one means of meeting the PCA requirement that there be significantly fewer variables than samples in a data set (Joliffe, 1986). We used the correlation matrix to standardize variables when measurement units differed or when coefficients of variation of more than a few variables were >1; otherwise, we used the covariance matrix in PCA (Joliffe, 1986; Legendre and Legendre, 1998). Results of PCA are presented as biplots, in which eigenvector loadings of each variable, usually scaled, are superimposed on a two-dimensional PC plot of the sample points (Gabriel, 1971).

3. Results

3.1. Effect of site on soil pH and resources

All sites have sandy soils with sand content being highest at Ocotillo (0.98 g sand g^{-1} soil) and decreasing with elevation; sand content at Laguna Summit is 0.78 g sand g^{-1} soil. Rainfall is lowest at Ocotillo (73 mm) and increases with elevation to 375 mm at Laguna Summit. As noted above, dominant plant species and percent cover were very different at the four sites. Site had a significant effect on soil pH and on all measured soil resources (Table 1), most likely due to a combination of differences in climate, soil type and plant community at each site. Both between (B) and under (V) plants, soil pH is highest at Ocotillo and Mountain Springs and, in general, declined with increasing elevation. At Ocotillo, total soil C and N between plants were below the detection limits for dry combustion. Otherwise, total soil C and N, and the C:N ratio increased with elevation for soils sampled between plants. The increases in MBC, MBN, C_{min35}, C_{min100} and N_{min} with elevation for soils sampled between plants were gradual, with the only significant differences between adjacent sites being between Jacumba and Laguna Summit. For soils sampled between plants pH is significantly lower and soil resources (except MBC) are significantly higher at the highest elevation, Laguna Summit, than at the two lowest elevation sites, Ocotillo and Mountain Springs.

For soils sampled under plants, the most pronounced differences among sites were increases in total soil C and N, and the C:N ratio with elevation. The increase in MBC and MBN with elevation was gradual. There was an increase in C_{min35}, C_{min100} and N_{min} between Ocotillo and Mountain Springs for soils sampled under plants but the only other difference between adjacent sites is that N_{min} was greater at Mountain Springs than at Jacumba. Soils sampled under plants at Laguna Summit have lower soil pH, and higher total C and N, C:N ratio and MBC than soils sampled under plants at both Ocotillo and Mountain Springs, but Laguna Summit soils have higher MBN, C_{min35}, C_{min100} and N_{min} than only the Ocotillo soils sampled under plants.

3.2. Effect of plants on soil pH and resources

We evaluated the influence of plants (fertile islands) on soil properties by comparing soils sampled between and under vegetation within each site (Table 1). In general, plants had no significant effects on soil pH, total C and N, and C:N ratio at any site. Although MBC and MBN tended to be higher in soils under plants than in soils between plants, these differences were statistically significant only at Laguna Summit for MBC and at Jacumba for MBN. A fertile island effect was most evident based on C- and N-mineralization values. Only at Laguna Summit were there no differences in C_{min35} and N_{min} in soils sampled between and under plants. C_{min100}, however, was different between and under plants at all sites. Differences in soil resources between vs. under plants were most pronounced at lower elevations where plants were spaced further apart, based on estimates of plant cover. No measurements were taken to evaluate the presence of roots in the open areas between plants.

A multivariate analysis of soil pH and resources among sites and locations shows that all sites and locations are unique (Fig. 1). The first and second principal components (PC1 and PC2) accounted for 91 and 8% of the total variance, respectively. Soil samples taken between plants are separated almost exclusively along PC1, which represents a gradient of high soil MBC, MBN, C_{min35}, N_{min}, total C, and total N, and low pH (Table 2). Soil samples taken under plants are also separated along PC1. Thus, in both cases, there is a clear pattern of increasing soil resources and decreasing pH with elevation. Samples taken under plants are also separated along PC2, with samples taken at Mountain Springs having the highest PC2 values. Separation along PC2 is due primarily to differences in N_{min} and C_{min35} among samples, as indicated by the relatively high PC2 eigenvector loadings for these variables (Table 2). At any given site, PC1 values are higher for samples taken under plants than between plants, illustrating a clear fertile island effect. Ocotillo and Mountain Springs samples taken under and between plants are also separated along PC2, due to large differences within these sites in N_{min} and C_{min35}. PCA patterns support the univariate data, in showing that the fertile island effect is strongest at the Ocotillo and Mountain Springs sites and weakest at Laguna Summit.

3.3. Effect of site and plants on FAME profiles

In total, 89 different fatty acids were extracted from the 23 soil samples analyzed. Individual FAME profiles were comprised of 6–62 FAME peaks, which were clearly separated on the chromatographs. We used the total number of FAMEs extracted from samples as a rough indicator of community richness and found that FAME number generally increased with elevation for soils sampled between plants but the only difference for soils sampled under plants is that FAME number at Ocotillo is lower than at Jacumba and Laguna Summit. We also found that when samples with MBC > 400 mg kg^{-1} are not included in the analyses, MBC is strongly correlated with FAME number ($r = 0.85$, $P < 0.0001$) and with FAME named ($r = 0.88$, $P < 0.0001$) and total areas ($r = 0.84$, $P < 0.0001$), as reported by the MIDI system. FAME number and areas for the four samples not included in the analyses (two samples taken under plants each at Jacumba and Laguna Summit) were not higher than for samples with ~200–400 mg kg^{-1} MBC. (When all samples are included, correlation coefficients for these same analyses are 0.67–0.70, $P \leq 0.0005$). One reason that more FAMEs are detected in samples with higher MBC is that FAMEs present in lower amounts are more likely to be present at

Table 1
Soil pH, C and N fractions, microbial community characteristics and ANOVA statistics for samples taken between and under plants at four sites in the Laguna Mountains

Site	Location[a]	pH	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	C:N	MBC ($mg\ kg^{-1}$)	MBN ($mg\ kg^{-1}$)	C_{min35} ($mg\ kg^{-1}$)	C_{min100} ($mg\ kg^{-1}$)	N_{min} ($mg\ kg^{-1}$)	Number of FAMEs	Number of Biolog substrates used
Ocotillo	B	8.1c	0a	0a	–	12a	1.1a	53a	140a	4a	8a	44a
	V	8.0bc	2.4a	0.4a	6.1a	56a	24.6ab	758b	1189cd	42cd	28bc	83d
Mt. Spring	B	8.1c	2.2a	0.4a	5.1a	115ab	18.2ab	208a	420ab	12ab	20b	47ab
	V	7.9bc	8.3ab	0.9b	8.8b	140ab	61.6bcd	1136c	2028ef	72e	36cde	80d
Jacumba	B	7.5b	12.8b	1.0b	12.0c	167ab	38.0abc	333a	820bc	27bc	36cde	51abc
	V	6.9a	16.3b	1.3b	12.9c	444bc	124.2d	1066c	2190ef	44d	40de	66bcd
Laguna Summit	B	6.9a	40.3c	2.3c	17.2d	342b	116.4d	916bc	1687de	49d	46e	84d
	V	6.8a	47.8c	2.6c	18.2d	575c	165.5d	1213c	2400f	34cd	41de	70cd
ANOVA statistics												
Overall *F*		14.6	31.6	34.3	46.1	10.5	12.4	22.2	19.1	15.1	16.9	5.4
Overall *P*		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.003
Site *F*		29.7	67.3	72.3	93.8	17.0	19.9	14.2	16	54	26.3	2.21
Site *P*		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.05	<0.0001	ns
Location *F*		5.9	4.7	10.6	20.0	10.5	14.5	91.4	71.1	39.0	17.7	13.6
Location *P*		<0.05	<0.05	<0.01	<0.0005	<0.01	<0.001	<0.0001	<0.0001	<0.0001	<0.001	<0.005
Site* location *F*		1.3	0.3	0.2	3.8	2.1	1.2	3.3	1.8	14.8	6.5	5.2
Site* location *P*		ns	ns	ns	<0.05	ns	ns	<0.05	ns	<0.0001	<0.005	<0.0116

[a] B = between plants; V = under plants.

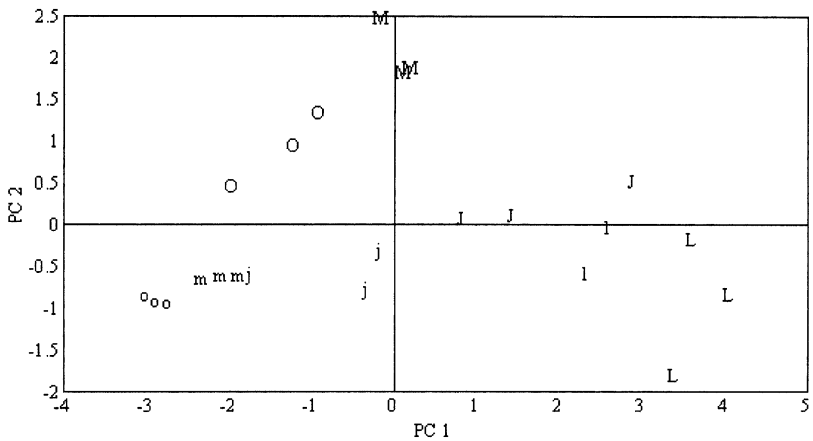

Fig. 1. PCA plot of pH and C and N fractions for soil samples taken between and under plants at four sites in the Laguna Mountains. m,M = Mountain Springs; o,O = Ocotillo; j,J = Jacumba Springs; l,L = Laguna Summit; small letters represent samples taken between plants and capital letters represent samples taken under plants. Eigenvector loadings are shown in Table 2.

levels above the GC detection limit in samples with high MBC than in samples with low MBC.

To account for significant relationships between FAME numbers and amounts and MBC we standardized our FAME data. We first divided the total named area of each sample by the named area of the subsample with the lowest total amount of FAMEs identified by GC. This procedure gave us a unique multiplication factor for each sample replicate. The peak area of each FAME within a profile was then multiplied by its respective factor. Those FAMEs whose resulting peak areas were lower than 500 (the set detection limit during our analyses) were removed from the data set because they represent FAMEs potentially present at levels below the detection limit in samples with low biomass. We were left with 39 total FAMEs after this standardization procedure. It is especially important to use some standardization procedure for data sets with a very wide range of MBC (such as our dataset) to ensure that differences identified among samples are not due to differences in microbial biomass size rather than microbial community structure. To meet the requirement that there be fewer variables than samples in a PCA, we further reduced our data matrix using two different strategies. Our first approach was to remove from the data matrix those fatty acids found in small quantities in only one of the analytical replicates. Removing these 14 FAMEs did not affect the pattern of samples in the first three dimensions of a PCA nor were any of these variables identified as having high loadings in a PCA conducted on the data set prior to their removal (data not shown). We used PCA conducted on the remaining 25 FAMEs to reduce the FAME data set even further. We decided to keep 14 FAMEs based on Kaiser's rule (Joliffe, 1986) and selected which 14 FAMEs to keep based on their eigenvector loadings in the first three PC dimensions (Cavigelli et al., 1995). Following each data reduction step we recalculated values to sum to 100% for each sample replicate. Then, we removed the variable with the lowest

Table 2
Eigenvector loadings for PCAs shown in Figs. 1–4

Variables analyzed (Fig.)	List of variables	PC1 loading	PC2 loading
Soil resources (Fig. 1)	MBC	0.40	−0.12
	MBN	0.42	−0.05
	C_{min35}	0.37	0.46
	N_{min}	0.22	0.78
	Total C	0.40	−0.29
	Total N	0.41	−0.18
	pH	−0.38	0.21
FAMEs (Fig. 2)	12:0	0.22	0.15
	16:0	−0.61	0.44
	18:0	−0.02	0.11
	i15:0	0.13	0.04
	i16:0	0.10	0.00
	i17:1 at 9	0.08	−0.06
	i17:1/a17:1	0.07	0.13
	i21:0	0.65	−0.07
	16:1 9c	−0.07	−0.21
	22:0 2OH	0.04	0.14
	22:2 13,16c	0.07	0.21
	18:2 9,12c/a18:0	−0.29	−0.79
Grouped FAMEs (Fig. 3)	Saturated straight chain	0.13	−0.23
	Saturated straight chain, >20C	0.15	−0.38
	Saturated cyclo	0.02	−0.04
	Saturated branching	0.38	0.84
	Monounsaturated	−0.89	0.23
	Polyunsaturated	0.07	−0.10
	Hydroxy 2	0.12	−0.08
	Hydroxy 3	0.07	−0.17
Biolog substrate groups (Fig. 4)	Starch polymers	0.33	−0.24
	4–5 C sugars	0.44	−0.32
	6 C sugars	0.20	0.67
	Dissacharides	0.29	−0.41
	Carbonic acids	0.31	0.22
	Amino acids	0.36	0.21
	Phospho compounds	0.28	−0.23
	Amides	0.42	0.29
	Aromatics	0.31	0.02

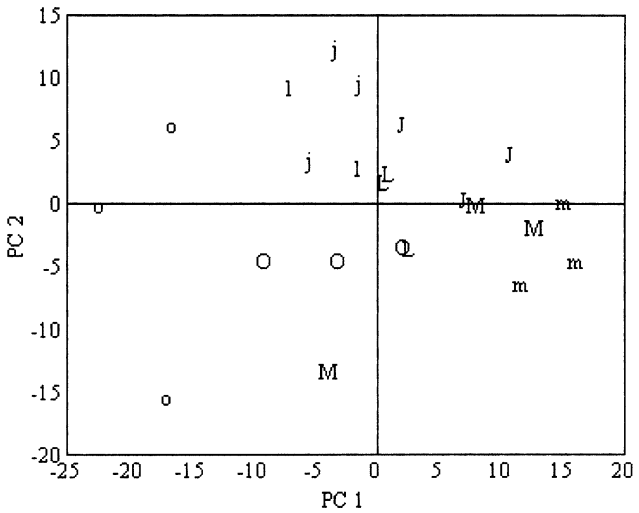

Fig. 2. PCA plot of 14 FAMEs for soil samples taken between and under plants at four sites in the Laguna Mountains. m,M = Mountain Springs; o,O = Ocotillo; j,J = Jacumba Springs; l,L = Laguna Summit; small letters represent samples taken between plants and capital letters represent samples taken under plants. Eigenvector loadings are shown in Table 2.

variance across all samples (17:1ω8c) to avoid singularity in the data matrix when all variables sum to 100% for each sample (C. Ramm, pers. comm.).

The PC plot resulting from PCA conducted on this 23 by 12 data matrix is presented in Fig. 2. PCs 1 and 2 accounted for 77 and 12% of total variability, respectively. Samples taken under plants tend to cluster closer to the origin and to each other than samples taken between plants, but there is no clear pattern among these four sample locations. Samples taken between plants tend to cluster closer to samples taken under plants at the same site than to samples taken under plants at different sites. This pattern is especially evident for the samples taken at Ocotillo and Mountain Springs. Samples taken between plants at Ocotillo have the most distinct FAME profiles. Samples taken between plants at Jacumba and Laguna Summit are similar to each other but both are different from samples taken at the same site but under plants.

Samples with high negative PC1 values are characterized as having proportionally high levels of 16:0, an ubiquitous fatty acid, and of the combined 18:2 9,12c/a18:0 (these two FAMEs are eluted from the GC column at the same time and are therefore not distinguishable with this method) (Table 2). Neither of these FAMEs is considered a biomarker. Samples with high positive PC1 values are characterized as having more Gram-positive marker FAMEs—the five Gram-positive marker FAMEs in this analysis (i15:0, i16:0, i17:1 at 9, i21:0, i17:1/a17:1) have positive loadings in PC1 (Table 2). Thus, samples taken between plants at Mountain Springs have proportionally high numbers of Gram-positive bacteria and samples taken between plants at Ocotillo, Jacumba and Laguna Summit have proportionally few Gram-positive bacteria. Samples with high PC2 values (some of the samples taken at Jacumba and Laguna Summit, including those sampled between plants) may have relatively higher eukaryotic biomass than other samples since 22:2 13,16c, a eukaryotic biomarker, has a relatively high PC2 loading. This FAME may be indicative of high fungal and/or plant biomass. There are no other indications, however, from this analysis that FAMEs of plant origin are responsible for differences among soil samples.

Our second approach to reduce the FAME data matrix was to group FAMEs into fractions as suggested by Zelles (1999). The results of PCA conducted on this data set are presented in Fig. 3. PC's 1 and 2 account for 84 and 7% of total variation. The clustering is similar to that shown in Fig. 2 in that samples taken between plants at Ocotillo cluster the furthest from all other samples. Monounsaturated FAMEs have the highest negative PC1 loading among these samples (Table 2). Since monounsaturated FAMEs are sometimes considered biomarkers for Gram-negative bacteria (Waldrop et al., 2000), this analysis suggests that these samples may be unique due to having a proportionally high

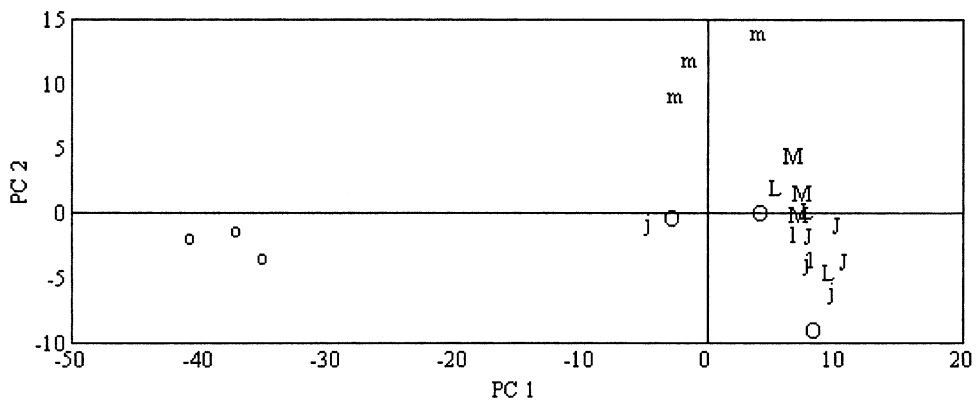

Fig. 3. PCA plot of FAMEs grouped by fractions for soil samples taken between and under plants at four sites in the Laguna Mountains. m,M = Mountain Springs; o,O = Ocotillo; j,J = Jacumba Springs; l,L = Laguna Summit; small letters represent samples taken between plants and capital letters represent samples taken under plants. Eigenvector loadings are shown in Table 2.

Table 3
Biolog substrates used in principal components analysis, grouped by type

Starch polymers	*Amino acids*
α-Cyclodextrin	D-Alanine
Dextrin	L-Leucine
Glycogen	L-Ornithine
N-Acetyl-D-galactosamine	L-Phenylalanine
4–5 C sugars	L-Threonine
i-Erythritol	L-Alanyl-glycine
Xylitol	Glycyl-L-glutamic acid
	γ-Amino butyric acid
6 C sugars	
L-Fucose	*Phospho compounds*
m-Inositol	D,L-a-Glycerol phosphate
D-Psicose	Glucose-1-phosphate
	Glucose-6-phosphate
Dissacharides	
Cellobiose	*Amides*
α-D-Lactose	Glucuronamide
Lactulose	Alaninamide
D-Melibiose	
Turanose	*Aromatics*
β-Methyl-D-glucoside	D,L-Carnitine
D-Raffinose	Urocanic acid
	Uridine
Carbonic acids	Thymidine
Mono-methyl succinate	Phenyl ethylamine
Acetic acid	Putrescine
Formic acid	
D-Galactonic acid lactone	
D-Glucosaminic acid	
D-Glucuronic acid	
α-Hydroxybutyric acid	
γ-Hydroxybutyric acid	
p-Hydroxy phenylacetic acid	
Itaconic acid	
α-Keto butryic acid	
α-Keto glutaric acid	
Malonic acid	
Propionic acid	
Sebacic acid	

Gram-negative bacterial biomass. Samples taken between plants at Mountain Springs are also distinct from samples taken under plants at the same site. As in Fig. 2, samples taken between plants at Mountain Springs have proportionally large amounts of branched saturated FAMEs (PC2 loading = 0.84, Table 2), which are Gram-positive biomarkers. Overlap among all other samples is fairly tight but again Laguna Summit samples show the least difference between samples taken between and under plants. Negative values in the PC2 dimension may be indicative of high eukaryotic populations since saturated FAMEs with greater than 20 C has the highest negative loading in this direction. Almost all samples from Jacumba and Laguna Summit have negative PC2 values. Since PC2 explains only 7% of total variation, these differences among samples may not be significant.

3.4. Effect of site and plants on C-substrate utilization profiles of soil microbial communities

To facilitate presentation and to meet the requirement that there be fewer variables than samples in a PCA, we classified the Biolog data (95 compounds) into nine chemical groups (Table 3). We first removed from the data set those Biolog substrates that were used by microbial communities from 20 or more samples, since these substrates are used by essentially all microorganisms and therefore provide little, if any, discriminating power. Then we removed all substrates that were used in only one analytical replicate in only a few samples. We grouped these remaining 51 compounds into the nine groups and used the proportion of individual substrates used in each group as the variables for PCA. The PCA plot of these data is presented in Fig. 4. PC1 accounts for 75 and PC2 for 8% of total variation. That all nine chemical groups had very similar loadings in PC1 (Table 2) implies that PC1 represents a gradient of all variables combined with samples having

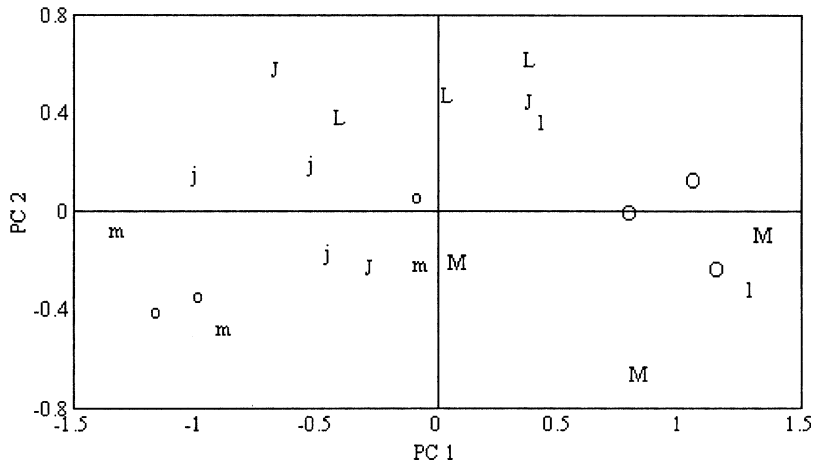

Fig. 4. PCA plot of Biolog substrates used, grouped by substrate type, for soil samples taken between and under plants at four sites in the Laguna Mountains. m,M = Mountain Springs; o,O = Ocotillo; j,J = Jacumba Springs; l,L = Laguna Summit; small letters represent samples taken between plants and capital letters represent samples taken under plants. Eigenvector loadings are shown in Table 2.

lower PC1 values being those with microbial communities able to use relatively few chemical groups. Thus, microbial communities under plants at Ocotillo and Mountain Springs used a greater number of compounds than those between plants at these same sites. At Jacumba there was no clear separation between samples taken between plants vs. under plants and at Laguna Summit microbial communities between plants tended to use more Biolog substrates than those under plants. To test whether the gradient of increasing substrate use along PC1 was related to MBC, we ran a correlation analysis between PC1 and MBC and found no significant relationship between these two variables, whether samples with MBC > 400 mg kg^{-1} were ($P = 0.15$) or were not ($P = 0.43$) used in the analysis.

Along PC2, samples with positive values harbor communities that are more likely to use 6 C sugars, amides, amino acids, and carbonic acids, while samples with negative PC2 values harbor communities that are more likely to use disaccharides, 4–5 C sugars, starch polymers and phospho compounds. Thus, microbial communities at Ocotillo and Mt. Spring tend to utilize more 6 C sugars, amides, amino acids, and carbonic acids than do microbial communities at Jacumba or Laguna Summit, regardless of whether they were below or between plants. But, since PC2 explains only 8% of total variation, these differences among sites and locations are small.

When comparing only samples taken between plants, substrate utilization profiles for communities at Ocotillo, Mountain Springs and Jacumba are relatively similar to each other while those at Laguna Summit are clearly different in that they use more compounds. This pattern is similar to other patterns (MBC, MBN, C- and N-mineralization) from Table 1. When comparing only samples taken under plants, Ocotillo and most Mountain Springs samples are again similar to each other but the Jacumba site is more similar to the Laguna Summit site.

4. Discussion

Soil properties were clearly influenced by both site and plants along the elevation transect in the Laguna Mountains of the western Sonoran Desert. From Ocotillo to Laguna Summit, elevation, precipitation and percent plant cover increased while sand content decreased. These factors probably all contribute to patterns of increasing soil C and N fractions and C- and N-mineralization rates and decreasing soil pH with elevation. Amundson et al. (1989) also found that soil C increased with elevation in the eastern Mojave Desert. Steinberger et al. (1999) found that soil microbial biomass increased along a desert climatic gradient. While we cannot allocate to each of these factors a specific effect on soil properties, climate certainly has strong direct and indirect influences on soil properties. Perhaps the strongest indirect effect of climate on soils is through plants. Plant density, measured using percent cover, increased with increasing elevation, allowing us to investigate the effect of plants on soil microbial communities and their activities.

Soil sample location (between or under plants) influenced every soil property we measured according to the ANOVA F statistic (Table 1). When comparing means within sites, however, only C- and N-mineralization rates were significantly influenced by the presence of plants. Surprisingly, other C and N fractions (total C and N, MBC and MBN) were, in general, not statistically different under vs. between plants. These patterns are in contrast with the findings of others who have consistently found higher total C, N, MBC, and MBN under vs. between plants in arid or semiarid climates (Charley and West, 1975; Crawford and Gosz, 1982; Parker et al., 1984; Garner and Steinberger, 1989; Kieft et al., 1998; Schlesinger and Pilmanis, 1998; Mazzarino et al., 1998; Xie and Steinberger, 2001). Our data do show, however, that all C and N fractions tend to be higher under than between plants at all four sites. Lack of statistically significant differences between vs. under plants may be due to the low number of samples taken at each location and site. The results of a multivariate analysis including six soil C and N fractions and soil pH, though, indicate that there is a clear fertile island effect at each site as defined by these seven parameters. This same analysis also suggests that the fertile island effect is strongest at lower elevations. The ANOVA analysis (site*location F statistic, Table 1) also indicates that the fertile island effect, as measured by C:N ratio, C_{min35} and N_{min}, is stronger at the lower elevations, where plants are spaced further apart than at the higher elevations.

We found that the number of FAMEs generally increased with elevation for soils sampled between plants but not for soils sampled under plants. The strong correlations we found between MBC and FAME number and areas are some of the first data showing there is a link between FAMEs and MBC. Thus, we conclude, as do Steinberger et al. (1999), using PLFAs, that microbial biomass and FAME number increase with rainfall in desert ecosystems. The link between FAME number and MBC also supports the need to standardize FAME data to ensure that differences in FAME profiles are not due to differences in microbial biomass.

The two different approaches we used to reduce the FAME data matrix provide two different perspectives. The first approach, reducing the number of FAMEs by keeping those that seem to have the most influence in distinguishing among samples highlights those individual FAMEs that most distinguish the different samples. The second approach, grouping FAMEs, has the risk of diluting any differences among samples by combining so many FAMEs into groups. Both approaches to reducing the FAME dataset, however, resulted in similar overall patterns that clearly identified soil samples taken between plants at Ocotillo as distinct from all others. These samples, based on biomarker analyses, may have low Gram-positive and high Gram-negative bacterial biomass relative to other samples. Both analyses also suggest that samples collected between plants

at Mountain Springs have higher Gram-positive bacterial biomass than do the other samples. In addition, FAME patterns under plants were relatively similar at all four sites, suggesting that plant presence is more important than plant species in affecting FAME profiles. That soils were different at each site does not affect this conclusion since the presence of plants had a convergent effect on FAME profiles relative to FAME profiles for the four sites when plants were not present.

The PCA plot of Biolog substrate data shows some similarities to the PCA plots of FAME data: soil microbial community physiological profiles between plants are most distinct from those under plants at both Ocotillo and Mountain Springs. These differences were clearly due to microbial communities under plants using more substrates than those between plants. Since Biolog PC1 values are not correlated to MBC, these differences are not due to differences in Biolog inoculation densities due to differences in microbial biomass that others have described (Waldrop et al., 2000; Fang et al., 2001). At Jacumba and Laguna Summit, differences between soils taken between and under plants were less distinct and were due to slight differences in the proportions of different substrate groups used.

It is not surprising that PCA of FAME and Biolog profiles provide slightly different patterns since the Biolog GN2 Plate we used select for Gram-negative bacteria while FAMEs are extracted from the soil microbial community as a whole. Thus, the Biolog plate provides a picture of the physiological profile of the community biased toward Gram-negative bacteria, possibly specifically Pseudomonads (Grayston et al., 1998). Although soils taken below plants at Ocotillo may have a greater proportion of Gram-negative bacteria than soils taken below plants at Mountain Springs and Jacumba (Fig. 3, Table 2), it seems that the physiological potential of these two communities is relatively similar (Fig. 4).

This is one of the few studies of FAME profiles in desert soils. Deserts provide several advantages for further study of the effects of plants on soil microbial communities. First, the influence of plants in desert soils is strong, as indicated by the existence of fertile islands. Second, many desert soils have low clay and organic matter content, two factors that can interfere with extraction procedures of molecular methods of describing soil microbial community structure and with the spectrophotometric aspect of the Biolog method.

Acknowledgements

Soil analyses were conducted at Michigan State University's W.K. Kellogg Biological Station, Hickory Corners, MI. We thank Mike Klug and Phil Robertson for use of their laboratories and the Center for Microbial Ecology, MSU, East Lansing, MI for funding. We thank Kathy Martin for help with laboratory procedures, Anne Conklin and Pete Krawczel for preparation of tables and figures, and Jeff Buyer for helpful comments.

References

Alpkem, 1992. The flow solution methodology. Nitrate + nitrite nitrogen and nitrite nitrogen and ammonia in seawater. Industrial method no's. P/N 000142, P/N 000143, and P/N 000672. Perstorp Analytical Instruments, Williamsburg, OR.

Amundson, R.G., Chadwick, O.A., Sowere, J.M., 1989. A comparison of soil climate and biological activity along an elevation gradient in the eastern Mojave Desert. Oecologia 80, 395–400.

Buyer, J.S., Drinkwater, L.E., 1997. Comparison of substrate utilization assay and fattyacid analysis of soil microbial communities. Journal of Microbiological Methods 30, 3–11.

Buyer, J.S., Roberts, D.P., Russek-Cohen, E., 2002. Soil and plant effects on and microbial community structure. Canadian Journal of Microbiology 48(11), 955–964.

Cavigelli, M.A., Robertson, G.P., Klug, M.J., 1995. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. Plant and Soil 170, 99–113.

Charley, J.L., West, N.E., 1975. Plant induced soil chemical patterns in some shrub-dominated semi-desert ecosystems of Utah. Soil Biology & Biochemistry 9, 357–365.

Crawford, C.S., Gosz, J.R., 1982. Desert ecosystems: their resources in time and space. Environmental Conservation 9, 181–195.

Elliott, E.T., Burke, I.C., Monz, C.A., Frey, S.D., Paustian, K.H., Collins, H.P., Paul, E.A., Cole, C.V., Blevins, R.L., Frye, W.W., Lyon, D.W., Halvorson, A.D., Huggins, D.R., Turco, R.F., Hickman, M.V., 1994. Terrestrial carbon pools: preliminary data from the Corn Belt and Great Plains regions. In: Doran, J.W., Coleman, D.C., Bezdicek, D.F., Stewart, B.A. (Eds.), Defining Soil Quality for a Sustainable Environment. Soil Science Society America Special Publication #35, American Society of Agronomy, Madison, pp. 179–191.

Fang, C., Radosevich, M., Fuhrmann, J.J., 2001. Characterization of rhizosphere microbial community structure in five similar grass species using FAME and BIOLOG analyses. Soil Biology & Biochemistry 33, 679–682.

Gabriel, K.R., 1971. The biplot graphic display of matrices with application to principal component analysis. Biometrika 58, 453–467.

Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology 57, 2351–2359.

Garner, W., Steinberger, Y., 1989. A proposed mechanism for the formation of 'Fertile Islands' in the desert ecosystem. Journal of Arid Environments 16, 257–262.

Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: Klute, A., (Ed.), Methods of Soil Analysis, Part I, second ed. American Society of Agronomy, Madison, WI, pp. 383–412.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology & Biochemistry 30, 369–378.

Harwood, J.L., Russell, N.J., 1984. Lipids in Plants and Microbes. Allen & Unwin, London.

Ibekwe, A.M., Kennedy, A.C., 1999. Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils. Plant and Soil 206, 151–161.

Jenkinson, D.S., Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil I. Fumigation with chloroform. Soil Biology & Biochemistry 8, 167–177.

Joliffe, I.T., 1986. Principal Component Analysis. Springer, Berlin, Germany.

Kieft, T.L., White, C.S., Loftin, S.R., Aguilar, R., Craig, J.A., Skaar, D.A., 1998. Temporal dynamics in soil carbon and nitrogen resources at a grassland–shrubland ecotone. Ecology 79, 671–683.

Legendre, P., Legendre, L., 1998. Numerical Ecology. Elsevier, Amsterdam, pp. 853.

MacMahon, J.A., Wagner, F.H., 1985. The Mojave, Sonoran and Chihauahaun Deserts of North America. In: Evenari, M., (Ed.), Hot Deserts and Arid Shrublands. Elsevier, Amsterdam, pp. 105–202.

Mazzarino, M.J., Bertiller, M.B., Sain, C., Coronato, F., 1998. Soil nitrogen dynamics in northeastern Patagonia steppe under different precipitation regimes. Plant and Soil 202, 125–131.

Noy-Meir, I., 1985. Desert ecosystem structure and function. In: Evanari, M., (Ed.), Hot Deserts and Arid Shrublands. Elsevier, Amsterdam, The Netherlands, pp. 93–103.

Parker, L.W., Santos, P.F., Phillips, J., Whitford, W.G., 1984. Carbon and nitrogen dynamics during the desomposition of litter and roots of a Chihuahuan Desert annual, *Lepidium lasiocarpum*. Ecological Monographs 54, 339–360.

SAS Institute, 2001. SAS User's Guide. Statistics. Version. 8. SAS Institute, Inc., Cary, NC.

Sasser, M., 2001. Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids. Tech. Note #101, MIDI INC. Newark, DE 19713.

Schlesinger, W.H., Pilmanis, A.M., 1998. Plant–soil interactions in deserts. Biogeochemistry 42, 169–187.

Siciliano, S.D., Theoret, C.M., de Freitas, J.R., Hucl, P.J., Germida, J.J., 1998. Differences in the microbial communities associated with the roots of different cultivars of canola and wheat. Canadian Journal of Microbiology 44, 844–851.

Sinsabaugh, R.L., Klug, M.J., Collins, H.P., Yeager, P.E., Peterson, S.O., 1999. Characterizing soil microbial communities. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, NY, pp. 318–348.

Steinberger, Y., Zelles, L., Bai, Q.Y., von Lutzow, M., Munch, J.C., 1999. Phospholipid fatty acid profiles as indicators for the microbial community structure in soils along a climatic transect in the Judean Desert. Biology and Fertility of Soils 28, 292–300.

Vestal, J.R., White, D.C., 1989. Lipid analysis in microbial ecology. Bioscience 39, 535–541.

Waldrop, M.P., Balser, T.C., Firestone, M.K., 2000. Linking microbial community composition to function in a tropical soil. Soil Biology & Biochemistry 32, 1837–1846.

White, D.C., 1983. Analysis of microorganisms in terms of quantity and activity in natural environments. In: Slater, J.H., Whittenbury, R., Wimpenny, W.M.T. (Eds.), Microbes in Their Natural Environments. Cambridge University Press, Cambridge, UK, pp. 37–66.

Xie, G., Steinberger, Y., 2001. Temporal patterns of C and N under shrub canopy in a loessial soil desert ecosystem. Soil Biology & Biochemistry 33, 1371–1379.

Zak, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G., 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biology & Biochemistry 26, 1101–1108.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils 29, 111–129.